Environmental Toxicology and Chemistry—Volume 00, Number 00—pp. 1–12, 2023
Received: 2 February 2023 | Revised: 10 April 2023 | Accepted: 12 April 2023

Critical Review

Studies of Endocrine Disruptors: Nonylphenol and Isomers in Biological Models

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Abstract: Certain emerging pollutants are among the most widely used chemicals globally, causing widespread concern in relation to their use in products devoted to cleaniness and asepsis. Nonylphenol ethoxylate (NPEOn) is one such contaminant, along with its degradation product, nonylphenol, an active ingredient presents in nonionic surfactants used as herbicides, cosmetics, paints, plastics, disinfectants, and detergents. These chemicals and their metabolites are commonly found in environmental matrices. Nonylphenol and NPEOn, used, are particularly concerning, given their role as endocrine disruptors chemical and possible neurotoxic effects recorded in several biological models, primarily aquatic organisms. Limiting and detecting these compounds remain of paramount importance. The objective of the present review was to evaluate the toxic effects of nonylphenol and NPEOn in different biological models. *Environ Toxicol Chem* 2023;00:1–12. © 2023 SETAC

Keywords: Endocrine disruptor; Environmental pollutant; Nonionic surfactants; Neurotoxic; Toxicology

INTRODUCTION

Nonylphenol is a toxic compound that is classified as a high-production-volume chemical (1 700 000 tonnes/year in the United States and 10 000 to 100 000 tons/year in the European Union); it is a potent endocrine disruptor and ecotoxic and has been detected in different environmental matrices. The main mechanism of nonylphenol-mediated toxicity involves the alteration of cellular redox homeostasis (Dwivedi et al., 2022). Importantly, nonylphenol is used for producing nonionic, nonylphenol ethoxylates (NPEOn) and other chemical products widely employed in industry and the home including textiles, pesticide formulations, paints, and cleaning agents (Hong et al., 2020). The NPEOns can enter wastewater treatment plants in substantial amounts, where they undergo biodegradation into various byproducts, including nonylphenol (Soares et al., 2008). Owing to their physicochemical characteristics, such as low solubility and high hydrophobicity, nonylphenol accumulates in environmental compartments like river water, seawater, soil, sediments, and aquatic organisms (Salgueiro-González et al., 2019), where it can be persistently detected (Zaytseva et al., 2020).

The NPs reach the environment through direct and indirect dumping of domestic wastewater and industrial plants (Bhandari et al., 2021). In addition, nonylphenol is ubiquitously persistent in food and drinking water, and nonylphenol consumption is considered a potential health risk to humans and biota. Several types of NPEOn are known to exist based on substitution of the hydroxyl group with a long chain of ethoxy groups, consisting of approximately 211 possible constitutional isomers, according to the production process (Hong et al., 2020). The NPEOn are classified as emerging pollutants owing to their widespread use, presence in ecosystems, and toxicity (Salgueiro-González et al., 2019). Other sources of pollution have been associated with these compounds, predominantly depending on their application, including oil and coal refineries and wastewater subjected to biological treatment in which NPEOn are partially degraded (Bhandari et al., 2021; Soares et al., 2008).

Degradation of NPEOn to nonylphenol in the environment involves microbial transformation by bacteria. The transformation of these compounds has been noted in natural aquatic systems and wastewater treatment plants; the products generated are considered refractory and more toxic than the original compounds. These compounds exhibit estrogenic effects in different biological models, including bivalves, nematodes, fish, and frogs (De la Parra-Guerra & Olivero-Verbel, 2020; De la Parra-Guerra et al., 2020). Documented

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DOI: 10.1002/etc.5633

effects of nonylphenol and its ethoxylates in the environment include feminization of aquatic organisms, reduced male fertility, and low survival of juveniles at concentrations of 8.2 μg/L (Kwack et al., 2002).

Owing to their harmful effects on ecosystems, the use and production of these compounds have been banned in the European Union and strictly controlled in several other countries, including Canada and Japan (Raecker et al., 2011). Nonylphenol is an endocrine disruptor capable of mimicking estrogen, a hormone related to sexual function (Noorimotlagh et al., 2020). As a xenohormone, nonylphenol interacts with the estrogen receptor (ER), and nonylphenol isomers can exhibit varying estrogenic potential. Moreover, nonylphenol was found to exert effects at the transgenerational level, with manifestations noted in the offspring of exposed organisms (De la Parra-Guerra et al., 2020).

The presence of nonylphenols and their isomers in the environment is considered significant, and detailed, robust, and comprehensive information is available on the toxicity induced by these compounds in different biological models (Li et al., 2018). To prepare the present review, we searched articles and performed data mining using the PubMed, Scopus, and ScienceDirect databases, by employing keywords such as "nonylphenol," "NPEOn," "contamination," "mechanism of toxicity," and "biological models." Similar results with historical information on the toxic effects of nonylphenol and NPEOn have been reported in different biological models. Globally, exposure to nonylphenols in biological models is critical for understanding their environmental kinetics; however, many studies are limited to the same compound, typically neglecting derivatives or isomers, which may exist as derivatives of the same molecule but display distinct properties (Li et al., 2018).

Ongoing ecotoxicological studies examining these pollutants have increased in recent years, revealing that nonylphenol, and its isomers are extremely toxic to aquatic life (long-lasting effects) and human health, presenting a considerable environmental risk (Tato et al., 2018). To investigate the action of emerging contaminants such as nonylphenol and NPEOn, it is necessary to employ suitable biological models with measurable parameters. Organisms that serve as structural or functional biomarkers are known to be altered when exposed to different concentrations of toxic substances present in environmental matrices (Tato et al., 2018). However, given the numerous applications of nonylphenol and related isomers, it is critical to review the use of these biological models and assess organism exposure, thereby identifying possible metabolic pathways induced by these contaminants on entering living organisms.

Our review provides an elaborate discussion of recent issues related to assessing the toxic effects of nonylphenol and NPEOn in diverse existing biological models, including different perspectives of recent research, particularly the role of an endocrine disruptor and its possible neurotoxic effects. We also elaborate on the conditions observed in several organisms, mainly aquatic, as well as their effects on the environment and related legislation.

PHYSICAL–CHEMICAL PROPERTIES OF NONYLPHENOLS

The term nonylphenol refers to a broad group of isomeric compounds ($C_{15}H_{24}O$) consisting of a nine-carbon alkyl chain bond linked to a phenol ring. Once it reaches the environment, 4-nonylphenol is the most generated and measured isomer. It is a viscous liquid at room temperature and can be a biodegradation product of alkylphenol polyethoxylate (Careghini et al., 2015), which is moderately bioaccumulative and undergoes photolysis in water (Martínez-Zapata et al., 2013). It also undergoes aerobic biodegradation in water, sediment, and soil systems, with high concentrations considered toxic to microorganisms (European Commission, 2002).

Nonylphenol has a molecular weight of 220 g/mol, a density of 0.953 g/mL at 20 °C, a dissociation constant of 10.7 ± 1.0, and an octanol/water partition coefficient (log K_{OW}) ranging from 3.8 to 4.8. The aqueous solubility of nonylphenol depends on the pH and temperature, and it is known to be soluble in several organic solvents. It exhibits an adsorption coefficient (K_d) that varies depending on the type of matrix (Düring et al., 2002). Consequently, given its relatively high adsorption, especially in soils and sediments, the mobility of nonylphenol is low (Jacobsen et al., 2004). Although the values for the nonylphenol derivatives decrease with an increasing number of ethoxy groups, the derivatives are more hydrophilic compounds (Düring et al., 2002).

Nonylphenol exhibits high lipophilicity (log K_{OW} of 5.76) and tends to be adsorbed on organic matter, which is an essential part of the sediment (Ding et al., 2019). Dissolved organic matter (DOM) is the main medium for nonylphenol transport in aquatic systems; it affords a high binding capacity for nonylphenol with its natural ligands and adsorption sites (Domene et al., 2009; Sadmani et al., 2014). In the presence of disturbances, DOM can diffuse from the interstitial water of the sediment toward the water columns, or it can be adsorbed on sediment particles, thereby resulting in the redistribution of pollutants (nonylphenol and ethoxylates) in the water bodies (Chen et al., 2018). Therefore, the migration range and rate of nonylphenol in aquatic systems can be expanded by binding at DOM (Ding et al., 2019).

The nonylphenol-9 isomer NPEOn has a molecular weight of 616 g/mol, a relative density of 1.05 g/mL at 20 °C, aqueous solubility, nine ethoxy units in its structure, and a K_{OW} ranging from 2.1 to 3.4 (Düring et al., 2002). These parameters form the basis for toxicity and bioaccumulation in the environment. Its presence in surface waters is mainly attributed to its application as a detergent, owing to its release via wastewater, industrial and domestic discharges, and effluents from treatment plants. Both nonylphenol and related ethoxylates are easily adsorbed on solid matrices, contaminating agricultural soils, where sludge is used as a fertilizer (Bhandari et al., 2021).

Surfactants

Surfactants, also known as surface agents, are a group of chemical compounds with numerous applications owing to properties such as solubility, detergent capacity, resistance

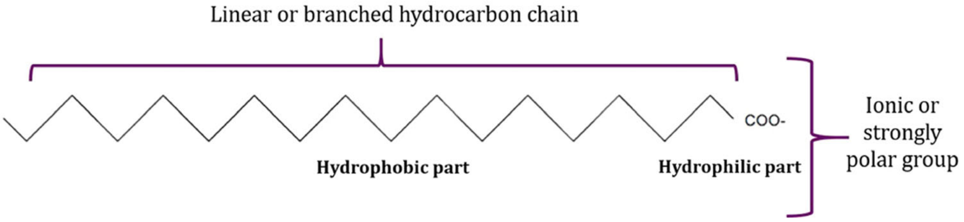

FIGURE 1: Basic structure of a surfactant.

to water hardness, emulsifiability, dispersibility, and humectant nature. Surfactants are amphiphilic in nature and consist of two structural parts or well-differentiated groups: a hydrophilic group (head group) and a hydrocarbon chain (hydrophobic tail; Figure 1). The presence of both these groups indicates that surfactants have various properties, such as the ability to decrease the surface tension of water, spreading or absorption of monolayers at the water/air interface, formation of emulsions and/or microemulsions, and micelle formation (Soares et al., 2008).

From a commercial standpoint, surfactants are classified based on their application. However, several surfactants exhibit various potential applications. Therefore, it is more appropriate to classify surfactants according to their molecular structure or their dissociation in water. Depending on the ionic character of the hydrophilic group, surfactants are divided into four major families: 1) anionic surfactants, 2) cationic surfactants, 3) nonionic surfactants, and 4) amphoteric surfactants (Ríos, 2016).

In the present study we have focused on nonionic surfactants, which are widely used in industry and have been minimally explored at the scientific level (Aronzon, 2012). This surfactant subfamily does not undergo ionization in an aqueous solution. In addition, it exhibits functional groups with high water affinity, is compatible with all surfactant types, has low foaming power, and can form liquid or pasty products. Ethoxylated alkylphenols are nonionic surfactants first synthesized in 1940, with a global annual production of 375 000 tons. The NPEOn have two critical groups: octylphenol ethoxylated (OPEO; production of 20%) and NPEOn (production of 80%). The chemical structure of NPEOn consists of an alkyl chain with nine carbon atoms, an aromatic group, and a polyethoxylated chain with a variable number of ethoxylated units (Figure 2). Increasing the length of the ethoxylated chain was shown to increase the specific weight, viscosity, and water solubility (Aronzon, 2012).

A typical NPEOn surfactant formulation is composed of nonylphenol, with an average of 10 ethoxy units, typically ranging between 1 and 50 ethoxy units (Li et al., 2018). There are widespread applications for NPEOn, but its resistance to biodegradation is a matter of concern in the environment. The generation of persistent metabolites, such as nonylphenol, has been identified in all types of natural water systems and wastewater, as well as in water for human consumption (Careghini et al., 2015). In addition to the negative properties, some metabolites, like nonylphenol, can mimic the female hormone 17β-estradiol by binding to the ER and competitively displacing 17β-estradiol (Acir & Guenther, 2018; Nice et al., 2003).

Therefore, nonylphenol and some short-chain isomers have been listed as endocrine disruptors and priority pollutants in the Water Framework Directive (Li et al., 2018), in the Canadian Environmental Protection Act (Government of Canada, 1999), and on the Contaminant Candidate List (CCL) of the US Environmental Protection Agency (CCL 4; 2021). Given their lipophilic structure, NPs and their isomers can bioaccumulate in adipose tissue, potentially reaching toxic concentrations (Noorimotlagh et al., 2020).

EXPOSURE AND TOXICITY OF NONYLPHENOL

The average daily intake of nonylphenols has been estimated as 0.5 µg/kg body weight for Chinese adults (Niu et al., 2015) and between 0.23 and 0.65 µg/kg in German children (Raecker et al., 2011). Moreover, it has been reported that continuous nonylphenol exposure through food or water intake can increase the toxicity of other endocrine-disrupting chemicals (EDCs; Zein et al., 2015) and markedly increase the risk of obesity, allergy, and breast cancer (Sprague et al., 2013). Significant concentrations of nonylphenols have been detected in several foods (0.1 and 100 µg/kg fresh wt), drinking water, and commercial beverages (Maggioni et al., 2013) across numerous countries, for example, in German supermarket-packaged foods (0.1 and 19.4 µg/kg fresh wt), shellfish and seafood in Asia, vegetables and fruits marketed in Sweden, Spain, and Florida (USA; Careghini et al., 2015), with approximate values of 5 and 50 µg/kg fresh weight, and significant values noted in carrots and pumpkins (10.4 and 11.3 µg/kg fresh wt, respectively), as well as in apples and citrus fruits (17.1 and 29.5 µg/kg fresh wt, respectively).

Based on data obtained from different studies, measured concentrations of nonylphenol in food, and expected consumption rates, the average daily intake of nonylphenol varies between 0.067 and 0.370 µg/kg/day for adults (60 kg body wt; Lu et al., 2013). In addition, the average daily intake of nonylphenol from bottled drinking water has been estimated to be 0.36–0.60 µg/day (Careghini et al., 2015).

The toxicity and potential neurotoxicity of nonylphenol and its isomers remain poorly characterized, and most studies have focused on assessing neuronal effects, especially in the offspring, using rodents as biological models (Noorimotlagh et al., 2020). Therefore, nonylphenol and its isomers present potential environmental risks owing to the induction of neurochemical and histopathological alterations following environmental exposure.

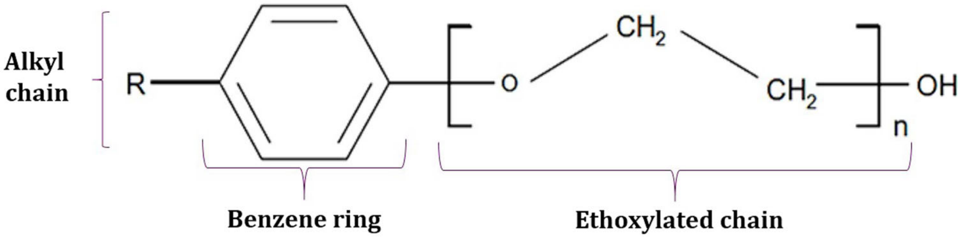

FIGURE 2: Basic structure of nonylphenol ethoxylate.

INVERTEBRATE MODELS

Balanus amphitrite

One of the first studies (by Billinghurst et al., 1998) on the effect of the environmental estrogen 4-nonylphenol was on the larvae of *Balanus amphitrite* exposed to concentrations of 0.01–10.0 μg L^{-1} for 24 and 48 h. Concentrations were determined using gas chromatography–mass spectrometry (GC–MS), revealing that larval sedimentation was significantly reduced. A natural estrogen, 17β-estradiol (10 μg L^{-1}), was employed as a positive control. The authors detected no endocrine alteration but a significant inhibition of larval sedimentation, indicating that 4-nonylphenol and 17β-estradiol exert toxic effects at real-world and natural environmental concentrations (Atienzar et al., 2002). Accordingly, it can be said that it resists endocrine alterations during the development stages. The validation of the model was done with a series of intra- and interlaboratory bioassays, allowing the use of *B. amphitrite* larvae as test organisms for ecotoxicological studies, complying with international precision standards both within and between laboratories (Abdulrahman et al., 2022). It should be noted that the endocrine disruption caused by this type of contaminant in these organisms is through the blocking or mimicry of the natural hormone in the specific binding site, because these contaminants generally have a chemical structure very similar to this hormone; thus the contaminant can enter the cytoplasm or cell nucleus without any problem from receptors.

Attempts are ongoing to develop techniques and procedures to significantly correlate endocrine disturbances attributed to water resources, especially those induced by marine environments in invertebrates, given the limited data available. The effects of exposure to the xenoestrogen, 4-nonylphenol have also been examined in marine crustaceans, particularly levels of a larval storage protein, cypris major protein, which is related to the vitellin of *B. amphitrite*, a dominant encrusting invertebrate in the marine environment (Matozzo et al., 2008). Larval and adult stages are considered an important model for examining the environmental pollutants that pose a potential threat to marine organisms and affect their ecosystems and coral reefs (Abdulrahman et al., 2022).

Bivalves as a biological model

We present a special section on biological models of bivalve species because, despite their ecological and economic relevance, data on bioaccumulation and contamination risks are lacking in terms of the consumption of certain edible bivalve species. Bivalves are considered one of the main matrices consumed by coastal communities worldwide and are classified as hyperaccumulators of pollutants (Mona et al., 2022). Studies using these biological models will help establish the risks associated with exposure to nonylphenol and its isomers and the appropriate consumer safety guidelines.

Crassostrea gigas. For evaluating the effect of nonylphenol on sperm motility in the Pacific oyster *C. gigas*, nonylphenol concentrations between 1 and 100 μg L^{-1} were used for 72 h, the timeline associated with gametogenesis. The nonylphenol exposure did not impact the growth rate of *C. gigas*; however, the concentrations examined significantly reduced motile spermatozoa ($p < 0.01$). Exposure to 4-nonylphenol for 72 h could impact the development of *C. gigas* during the larval stage, delaying embryonic development (Nice, 2005).

Coastal marine ecosystems are constantly exposed to synthetic and natural chemicals from residential, industrial, and agricultural activities on land, further exacerbating pollution. In terms of possible toxicants, the ubiquitous aquatic xenoestrogen nonylphenol has implications for reproduction, development, growth, and, more recently, immune function in marine invertebrates. Short-term (7-day low [2 μg L^{-1}] and exposure to ecologically relevant concentrations) and long-term exposure can alter cellular and humoral elements of the innate immune response in *C. gigas*, an aquaculture species of global economic importance (Hart et al., 2016). Simultaneous exposure to chemicals could increase disease incidence or severity, impacting marine biodiversity as well as health and food safety, especially seafood (shellfish and fish) grown commercially in these environments; accordingly, their consumption can lead to an increase in toxicity to humans (Guéguen et al., 2011). Reportedly, *C. gigas* can accumulate significant levels of linear alkylbenzenes, similar chemicals, or nonylphenol-related compounds, particularly at sites contaminated with sanitary wastewater, exhibiting reduced biological responses (Flores-Nunes et al., 2015).

Both nonylphenol and its related isomers are known surfactants classified as endocrine disruptors; they are considered new contaminants, and public and scientific concerns have been growing in recent decades, given their small size and potential impact on human and ecological health. Elucidating the toxicological effects of these pollutants on marine organisms remains of considerable importance; however, most previous research studies have employed standardized model organisms at the laboratory level. There is a gap in knowledge about the distinct biologies of marine biodiversity (Gao et al., 2022).

Cerastoderma glaucum. In the bivalve *C. glaucum*, the lethal and sublethal effects of 4-nonylphenol were evaluated by performing a 96-h lethality test, with a median lethal concentration (LC50) of 0.3 mg/L at 96 h, and no mortality at 0.1 mg/L. The 4-nonylphenol exposure significantly increased alkaline phosphatase levels in the digestive gland of males (at all concentrations) and females at 0.025 and 0.1 mg/L. These findings indicate that nonylphenol induces vitellogenin (Vg) synthesis in *C. glaucum* while revealing the higher 4-nonylphenol sensitivity of males than that of females. Exposure to 4-nonylphenol has been shown to adversely impact *C. glaucum* (Marin et al., 2008).

Exposure to nonylphenol and its isomers also altered the functional response of *C. glaucum* haemocytes (blood cells), primarily by reducing cell membrane stability and promoting cell degranulation. A fraction of hemocytes, approximately 7–8 μm in diameter and 250 L in volume, was markedly increased in *C. glaucum* exposed to high concentrations of nonylphenol (0.1 mg/L). Importantly, exposed animals exhibited apoptosis resulting in reduced cell volume and increased acid phosphatase activity in cell-free hemolymph (Matozzo et al., 2008).

Mytilus galloprovincialis. Similar studies were performed in *M. galloprovincialis*, with organisms exposed for 7 days to 4-nonylphenol. Bioaccumulation of 4-nonylphenol in soft tissues and levels of vitellogenin-like protein (Vg) in the digestive glands of males and females were measured by the alkaline labile phosphate assay. Exposure induced Vg synthesis in male and female mussels, which may interfere with the endocrine system, disrupting the normal hormonal functions of the organism. Nonylphenols can mimic the action of endogenous estrogens by binding to estrogenic receptors and leading to the induction of Vg synthesis (Ricciardi et al., 2008). In general, Vg induction is a biomarker of exposure to estrogenic compounds, such as nonylphenol, validated in mussel models (Marin et al., 2008), and has been successfully used as an indicator of the presence of xenoestrogens in environment.

Spain is considered the main producer of *M. galloprovincialis*, a bivalve of economic interest in aquaculture. In *M. galloprovincialis*, exposure to nonylphenols (0.054–0.103 ng/L) and other contaminants could impact metabolic pathways involving carboxylesterases (CEs), which are α- and β-hydrolase–folding proteins catalyzing the hydrolysis of a wide range of endogenous and exogenous compounds. These findings suggest that CE inhibition may be an adequate biomarker of contaminant exposure, which could be incorporated into a battery of subindividual indicators of toxicity exposure (Solé & Sanchez-Hernandez, 2018). The role of this metabolic enzyme has been well established in pesticide detoxification, therapeutic drug metabolism, and anticancer prodrug activation.

Both nonylphenol and its isomers are among the most frequently detected organic contaminants in wastewater, with a high environmental risk in coastal marine ecosystems, as indicated by the concentrations exposed to *M. galloprovincialis* in Tato et al., 2018. In addition, *M. galloprovincialis* is considered a moderate nonylphenol bioacumulator in water, with a bioconcentration factor of 6850 L kg^{-1} (dry wt), which also significantly inhibited acetylcholinesterase activity and induced glutathione S-transferase (GST) and glutathione peroxidase (GPx) activities. The GST induction was dose dependent, whereas the GPx activity exhibited a less consistent pattern; however, in both cases, the induction was maintained after a 10-day clearance period (Vidal-Liñán et al., 2015).

It should be noted that emerging pollutants (endocrine disruptors) are chemicals yet to be incorporated into regulatory control programs, whose fate and biological impact remain poorly clarified. Therefore, the impact of these chemicals on the health of ecosystems should be explored. The marine environment is of particular concern, and data regarding the impact on early life could establish the sensitivity of marine species to these contaminants. In *M. galloprovincialis*, larval stages have been examined to establish the impact of contaminants on development, with specific methodologies developed for this purpose (Fabbri et al., 2014).

Drosophila melanogaster

Water pollution due to human activities can result in sedimentation, excess nutrients, and toxic chemicals, impacting the normal endocrine function of living beings. The fruit fly is a standardized biological model widely utilized in molecular biology, biochemistry, toxicology, ecotoxicology, and genetics, owing to its benefits as an organism and gene homology with human beings. Water contamination induced by nonylphenol (1670.9 μg/L ± 837.6 g/L = mean ± 1 SD of nonylphenol) can impact the health (development time and fertility) of *D. melanogaster*, especially the stage from pupa to adult and even at the transgenerational level. However, in some cases, flies could develop different strategies to circumvent various stressful environmental factors (Quesada-Calderón et al., 2017).

In *D. melanogaster* larvae exposed to nonylphenol (0.05, 0.5, and 5.0 μg/mL), messenger (m)RNA levels of heat shock protein 27 (Hsp27) and ecdysone receptor (EcR) were reduced in the midgut. Simultaneously, levels of reactive oxygen species (ROS) were found to be increased, along with a corresponding decrease in glutathione (GSH) levels and thioredoxin reductase (TrxR) activity and increased lipid peroxidation (LPO), protein carbonyl (PC) content, and cell death. Collectively, overexpression of Hsp27 in *D. melanogaster* midgut cells could reduce the nonylphenol-induced intracellular content of ROS, LPO, and PC, and could also reduce cell death through the TrxR-mediated regenerative pathway as well as GSH levels, thereby improving the body's response to exposure. The fertility of the fly is another parameter impacted by exposure to these contaminants, inducing an imbalance in the developmental ecology of these organisms and classifying nonylphenol and its isomers as genotoxic contaminants (Dwivedi et al., 2022).

In addition, acute exposure to sublethal concentrations of a nonionic surfactant (polyethoxylated tallowamine) demonstrates the affectation in the *D. melanogaster* model, on carbonyl protein levels, marked inhibition of carbonyl reductase activity, and reduction in fecundity (by cell viability through improved apoptosis), thus confirming the toxicity that these groups of surfactant contaminants can have in ecosystems and especially in different organisms ř(Bednářová et al., 2020). Some studies have observed toxicity of polyethoxylated tallowamine in fish, mammals, and human cells, even exceeding the toxicity of other toxins such as glyphosate ř(Bednářová et al., 2020; Chłopecka et al., 2017; Mesnage et al., 2013).

VERTEBRATE MODELS

Cyprinus carpio

Classical toxicological parameters, such as hematological parameters and histopathological alterations, have been examined in the *C. carpio* fish model. The toxic effects of sublethal nonylphenol concentrations of environmental relevance were examined, and juveniles exposed to nonylphenol concentrations of 1–15 μg/L for 70 days predominantly exhibited hematological effects. Severe anemia was detected, with no histopathological alterations in the tissues of exposed fish. Based on these important findings, the general toxic effects of nonylphenol might outweigh its weak estrogenic effects, potentially disrupting ecological processes such as fish reproduction under field conditions (Schwaiger et al., 2000). Notably, in studies assessing nonylphenol toxicity and derivatives, data regarding transgenerational effects have been reported (Nice et al., 2003).

Studies have shown that nonylphenol exposure-induced mortality, growth retardation, hepatorenal dysfunction, suppression of immune antioxidants, histopathological degeneration, and negative regulation of genes can be evaluated in the same model (Abdel Rahman et al., 2022). Extensive pathological studies have revealed that nonylphenol can damage hepatocytes and renal epithelium and induce monocellular cell infiltration in the hepatorenal tissues of fish. The nonylphenol isomer 4-nonylphenol, also known as an aquatic microchemical contaminant, could damage the reproductive parameters and histology of *C. carpio*; concentrations of 10, 50, and 100 μg/g body weight notably increased plasma progesterone and Vg in both males and females, whereas the level of testosterone decreased significantly. In addition, the presence of severe histopathological changes was noted in the ovaries, testes, and liver of fish (at concentrations of 10, 50, and 100 μg/g body wt). Accordingly, classic 4-nonylphenol has the potential to induce hepatotoxicity and gonadotoxicity (Amaninejad et al., 2018). In general, this family of surfactants appears to be markedly toxic to different species of fish, damaging gill morphology, inducing mucus excretion from these organs, as well as lipid peroxidation (Gheorghe et al., 2022) and hormonal and sexual alterations. These findings indicate the importance of persistently monitoring the nonylphenol family of contaminants and establishing their complete cycle in aquatic ecosystems.

Oncorhynchus mykiss

The estrogenic effects of nonylphenol were examined in rainbow trout fish (*O. mykiss*), considering sexual maturity and possible transgenerational alterations in their offspring. In directly exposed male rainbow trout, nonylphenol was shown to act as a weak estrogen, as determined by the elevated plasma Vg levels and reduced reproduction and hatching rates. The hormonal imbalances detected in the offspring of exposed fish indicated the occurrence of a transgenerational effect mediated by the endocrine system, a notable finding in this field (Schwaiger et al., 2002). In addition, studies have shown that 4-nonylphenol exposure at concentrations of 130 ng/L or more significantly reduced semen production in this species when compared with unexposed fish, whereas sperm density, motility, and semen fertility were not affected. In addition, 4-nonylphenol also affected egg viability, sperm motility, and fertilization rates (Lahnsteiner et al., 2005).

Shelley et al. (2012) exposed rainbow trout to NPEO2 for 72 h. Fish were euthanized, the gallbladder was harvested, and radioactivity was measured in tissues and viscera. Fish exposed to high concentrations of nonylphenol (18 μg/L) displayed physiological changes, significantly elevated liver somatic index, changes in gene expression, Vg induction consistent with estrogenic effects, reduced lymphocytes in peripheral blood, and alterations in immune-related pathways in the liver transcriptome (Shelley et al., 2012).

le Gac et al. (2001) examined the biotransformation of NPEO2 in vivo and in vitro in rainbow trout. The major metabolite present in bile corresponds to NPEO2-glucuronide; however, this metabolite was not detected in vitro. Accordingly, hepatocytes may display a different metabolic pattern in whole fish while exhibiting evidence of an unknown metabolic pathway grasp in vivo. In addition, the effects of NPEO2 on spermatogenesis were examined both in vivo and in vitro. The highest concentrations of NPOE2 (225–970 nmol/L) significantly increased blood plasma levels of Vg during the maturation of juvenile males. Fish at the initial stage of spermatogenesis were exposed to 580 nmol/L of NPOE2 for 21–27 days. A 20%–40% reduction in the gonadosomatic index was noted 4.5 weeks after exposure, and the process of spermatogenesis was partially inhibited (le Gac et al., 2001); other alterations produced by mixtures of nonylphenols were also observed, thereby establishing the risk of the mixtures on aquatic organisms (Crago et al., 2015).

In addition, NPEO2 could inhibit multidrug-resistant 1 (MDR-1) activity in vitro, but did not alter in vivo hepatic levels of P-glycoprotein (P-gp) in exposed trout. The tissue distribution of P-gp plays an important role in defending organisms against xenobiotics, reducing their absorption and excretion. In terms of the interaction of P-gp, in the liver and hepatocytes in primary culture, with NPEO2, exposure to sublethal NPEO2 concentrations failed to induce any change in hepatic P-gp levels in juvenile trout. This finding demonstrated that NPEO2 inhibited MDR-1 in trout hepatocytes, suggesting that water contaminants may interfere with the function of P-gp in fish, potentially impacting the body's defence against xenobiotics (Sturm et al., 2001).

Finally, external factors, such as environmental contaminants in sediments, should be of concern, given that several EDCs accumulate in this matrix, and minimal data are available regarding the bioavailability and effects of these EDCs in association with sediments in the environment. For instance, chemicals are released from sediment during floods and become bioavailable to aquatic biota (Müller et al., 2021).

Danio rerio

Zebrafish (*D. rerio*), another laboratory-scale aquatic model, has been widely used to assess multiple pollutants, including estrogenic molecules such as nonylphenol (99% GC, mixture of isomers; Acros) and compounds capable of inducing Vg production in fish. Experimental results have demonstrated the suitability of in vitro assays for qualitative evaluation of compound estrogenicity, while highlighting the need for further in vivo assays (van den Belt et al., 2004). In fish, 4-nonylphenol enters through the gills to induce its estrogenic potential, as evidenced in two experiments, one providing 4-nonylphenol in water and the other supplying 4-nonylphenol in the diet. Fish exposed to 4-nonylphenol through water showed a 10-fold greater sensitivity than fish exposed orally. This finding indicates that 4-nonylphenol (mean values of 0.93 ± 0.1 μg/L) exhibits potent estrogen potential, which is further enhanced when the contaminant enters the bloodstream through fish gills rather than via dietary exposure (Pickford et al., 2003).

Studies have demonstrated the toxic responses to nonylphenol and its isomers during development, as well as in the nervous system and reproductive parameters of several animals (Mukherjee et al., 2022). The zebrafish model has allowed the determination of liver pathophysiologies, especially at the relevant environmental concentrations, assessing hepatic redox homeostasis in relation to cellular energy sensors, inflammatory response, and cell death. The nonylphenols could promote ROS synthesis and lipid peroxidation, indicating homeostasis-deregulated energy, metabolic alteration, and macrovesicular steatosis, as well as, in turn, chronic inflammation and hepatotoxicity in exposed males (Mukherjee et al., 2022), as determined in the liver, the largest endocrine organ involved in detoxification (Yu et al., 2018).

Biomarkers are commonly used to evaluate and analyze the toxic effects of several environmental pollutants. In zebrafish, antioxidant enzymes are commonly used as biomarkers, which are complex under environmental situations, particularly in the real-world environment contaminated with substances in the water, modifying the biological effects of endocrine disruptors and impacting their adsorption, transport, enrichment, and even their induced toxicity. Studies have revealed these types of nonylphenol-mediated interactions and other alterations (Qian et al., 2018). Table 1 summarizes the main effects of nonylphenol in different invertebrate and vertebrate biological models.

Oryzias latipes

The biological model known as Japanese medaka (*O. latipes*), has been widely used to assess toxicity by 4-nonylphenol and other groups of surfactants; this model has contributed to the biochemical responses arising from this type of contaminant (Kawashima et al., 2022; Watanabe et al., 2017). Through proteomic analysis, *O. latipes* exposed to different concentrations of cationic surfactants (used as disinfectants) displayed significant increases in the oxidative stress response, nervous and endocrine systems, signaling pathways, and cellular proteolysis (Kwon et al., 2020). Therefore, risk assessments have been made by means of ecological indices that involve different aquatic models, to evaluate the variations in different parameters of the aquatic ecosystem on the toxicity of surfactants in aquatic species, of which this model plays a fundamental role.

In the Japanese medaka model, 4-nonylphenol-induced erythrocyte alterations, apoptosis, and micronuclei have been determined after exposure; many morphological alterations and nuclear abnormalities were observed in the present investigation, including acanthocytes, lobulated nucleus, eccentric nucleus, fragmented nucleus, blistered nucleus, binuclei, deformed nucleus, notched nucleus, hemolyzed cells, crenate cells, teardrop-shaped cells, schistocytes, and *O. latipes* could also be classified as a sensitive experimental model of excellent use in ecotoxicology to assess genotoxicity (Sayed et al., 2018).

EFFECTS OF NONYLPHENOL IN THE ENVIRONMENT

Accumulating evidence provides growing concern regarding nonylphenol contamination, given its persistence in ecosystems, bioaccumulation in biota, and toxicity to organisms (Dwivedi et al., 2022). Given the rapid rate of urbanization, modernization, and industrialization, a considerable amount of nonylphenols has gained access to water sources in recent decades, including rivers, lakes, and reservoirs (Hong et al., 2020). Through the several adverse and toxic effects on humans and the environment that nonylphenol reportedly exerts (Bhandari et al., 2021), it can reduce male fertility in aquatic organisms, as well as the survival of juveniles at concentrations of 8.2 $\mu g\,L^{-1}$ (Bhandari et al., 2021; Yang et al., 2020). Moreover, nonylphenol was shown to be acutely toxic to phytoplankton, zooplankton, amphibians, invertebrates, and fish (Bhandari et al., 2021; Hong et al., 2020).

The possible nonylphenol-mediated toxic effects on biota and humans include endocrine disruption, pollution, feminization of aquatic organisms, allergic reactions, bioaccumulation, mutations, and cancer (Bhandari et al., 2021; Dwivedi et al., 2022).

It is well recognized that nonylphenol toxicity is mediated via its ability to interact with estrogen (Soares et al., 2008), given its structural similarity to the phenolic ring with 17β-estradiol, thereby impacting the development, maintenance, and functions of female reproductive organs, cycles of sexual activity, and secondary female sexual characteristics. The structural similarities between nonylphenol and 17β-estradiol are schematically illustrated in Figure 3.

Similarly, nonylphenol and its derivatives can markedly impact the cardiovascular system and blood clotting. Mueller and Kim (1978) first reported the endocrine disrupting activity of nonylphenol, which was subsequently confirmed by Soto et al. (1995). Since then, numerous studies have shown the actions of nonylphenols as endocrine disruptors and their effect on activating the estrogen receptor α, which induces the proliferation of cancer cells (Noorimotlagh et al., 2020).

TABLE 1: Summary of nonylphenol effects in different invertebrate and vertebrate biological models

Route of exposure	Concentration	Evaluated endpoint	Animal model	Reference
Intraperitoneal route	NP (25 mg/kg), for 21 days	NP-induced memory deficit, neurotoxicity	Male Wistar rats	Lotfi et al., 2022
Alimentation	NP (500 µg/L) for 4 weeks	Effects of NP on mammalian oocyte quality examined from mouse organelles	ICR mice	Hu et al., 2022
NP dissolved in water	NP (1, 10, and 100 µg/L) for 21 days	Effects of NP on plasma levels of reproductive hormones and hepatic antioxidant enzymes, histopathology of male and female reproductive and nonreproductive organs	Caspian brown trout (*Salmo trutta caspius*) smolts	Shirdel et al., 2020
NP dissolved in water	NP (0.1 mg/kg body wt) for 3 weeks	Histophysiological effects in fish exposed to NP	African catfish (*Clarias gariepinus*)	Abd-Elkareem et al., 2018
Cell incubation	Incubation with L-15 medium containing NP (0 [control], 10–5.2 × 10–5.3 × 10–5 mol/L), for 6, 12, and 24 h	Toxic effects of NP on the antioxidant defence system (measurement of catalase, superoxide dismutase, glutathione peroxidase, lipid peroxidation, total antioxidant power, and total protein)	Liver cells obtained from (*Liza klunzingeri*)	Salamat & Derakhshesh, 2020)
Water treatment	NP (50 µg/L of water; equal to 1/28 of LC50–96 h) for 3 weeks	To examine the possible favorable role of zinc sulfate against the immunosuppressive, hepatotoxic, and nephrotoxic effects of NP	Nile tilapia (*Oreochromis niloticus*)	Mohamed et al., 2019
Fed on a diet	High-fat, high-sucrose diet (HSHFD) combined with NP at doses of 0.02 µg/kg/day (NP-L-HSHFD), 0.2 µg/kg/day (NP-M-HSHFD) and 2 µg/kg/day (NP-H-MSHFD), for 90 days	Effect of EED-NP on nonalcoholic fatty liver disease in rats fed an HSHFD	Sprague-Dawley rats	Yu et al. (2018)
Exposure in neonates	Stock solution 4-NP (15 µg/mL). Final concentrations 4-NP (0.6, 3, and 15 µg/L) during 24 and 48 h	Analyzed the transcriptional modulation of 11 potential molecular indicators related to detoxification, oxidation, development, and cell stress in *Daphnia magna*	*Daphnia magna*	Kim et al., 2019
Feeding in the aquarium	First two groups were exposed to 100 and 500 µg/L of NP, respectively	Change in transcriptional expression of genes related to the immune system and variation of antioxidant enzyme activities after exposure to NP in a mollusc to explore the immunomodulatory capacity of NP	*Chlamys farreri*	Liu et al., 2022
Oral treatment	NP (100 µg of NP/prawn) for 1, 3, 6, and 9 days	Effects of NP on susceptibility to a pathogen and mRNA expression of hemocyte genes, including four genes related to the immune system	Prawns (*Macrobrachium rosenbergii*)	Sung & Ye, 2009
Treatment in algae culture	NP concentrations (0, 0.5, 1, 1.5, 2, and 3 mg/L) for *C. pyrenoidosa* Concentrations of microplastics and NP in combined toxicity were 50 and 2 mg/L during 96 h	Influence of the combined toxicity of NP with three types of microplastics containing polyethylene and polystyrene on microalgae	Microalgae (*Chlorella pyrenoidosa*)	Yang et al. (2020)
Oral treatment	NP (10–500 µM) and NP-9 (150–100 000 µM), for 12, 24, and 72 h	To determine the toxicity of NP and NP-9 in different endpoints in *Caenorhabditis elegans*	Nemátode (*Caenorhabditis elegans*)	De la Parra-Guerra & Olivero-Verbel (2020)
Oral treatment	Technical mixture of NPEO containing chain isomers and oligomers with an average of 8 ethoxy units (NP8EO); technical grade 4-nonylphenol with a purity of 95% was also used	Provide the range of concentrations of NP and nonylphenol ethoxylates NP1EO and NP2EO) with ecotoxicological effects on different plants and soil invertebrate species	Plants, earthworms, enchytraeids, and springtails	Domene et al. (2009)

(*Continued*)

TABLE 1: (*Continued*)

Route of exposure	Concentration	Evaluated endpoint	Animal model	Reference
Oral treatment	The stock solution (10 mM) was prepared using Milli-Q water, and subsequent dilutions were made with M9-buffer	Evaluate the intergenerational toxicity of NP-9 in *C. elegans*	Nemátode (*Caenorhabditis elegans*)	De la Parra-Guerra et al. (2020)
Liquid exposure of yeast cells	NP (7 and 60 mg/L)	Evaluate the genotoxicity of 4-NP and NP ethoxylate (NpnEO) using *Saccharomyces cerevisiae* strain D7 as an experimental model	Fungus (*Saccharomyces cerevisiae*)	Frassinetti et al., 2011
Incubation in treated seawater	The most toxic compound tested was 4-NP, with an effective concentration of 10% at 11.1 µg L^{-1} for *Isochrysis galbana*, 110.5 µg L^{-1} for *Mytilus galloprovincialis*, 53.8 µg L^{-1} for *Paracentrotus lividus*, and 29.0 µg L^{-1} for *Acartia clause*	To assess the environmental risk of three phenolic compounds of special environmental and human health concern used in plastics and household products: bisphenol A (BPA), triclosan (TCS), and 4-NP	Microalgae (*Isochrysis galbana*), mussel (*Mytilus galloprovincialis*), sea urchin (*Paracentrotus lividus*), and the copepod (*Acartia clausi*)	Tato et al. (2018)

EED = environmental endocrine disruptor; ICR = Institute for Cancer Research; LC50 = median lethal concentration; NP = nonylphenol.

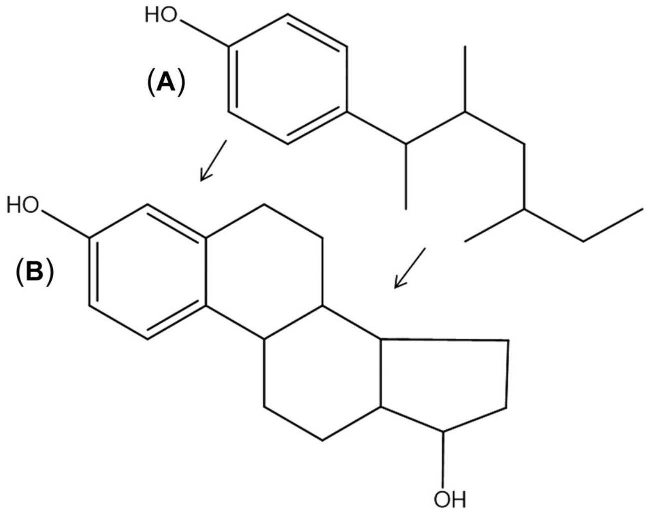

FIGURE 3: Similarities in the chemical structure of nonylphenol (NP; **A**) and 17β-estradiol (**B**).

LEGISLATION REGARDING THE USE OF NONYLPHENOL

It is estimated that approximately 60% of nonylphenols and their derivatives manufactured worldwide enter water supply systems (Bhandari et al., 2021; Hong et al., 2020). Given their broad spectrum, some European countries have started restricting the application and production of nonylphenol (Bhandari et al., 2021; Soares et al., 2008).

Owing to concerns regarding the use of nonylphenols and NPEOns, some countries have created regulations to determine the maximum permissible value of these contaminants in surface water bodies and sewers. At the international level, few countries have established relevant regulations. Considering existing laws, the European Directive 2003/53/EC (Directive 2003/53/EC, 2003) has been established, with certain restrictions imposed on the marketing, use, and preparation of nonylphenols and NPEOn. In addition, the European Union advises limiting the concentrations of nonylphenols and NPEOn in sludge to 50 mg/kg (Working Document on Sludge 2000). Denmark and Switzerland have legislation regarding the concentrations of nonylphenols and NPEOn present in agricultural soils, with maximum values of 10 mg/kg in Denmark and 50 mg/kg in Switzerland (European Commission, 2002). Given the scarcity of laws regulating the presence of these pollutants in environmental matrices, high concentrations of nonylphenols and NPEOn have been documented in sediments, soils, waters, sludge, and food (Guenther et al., 2002).

In Brazil, the National Environment Council (Conselho Nacional Do Meio Ambiente, through Resolution [Conama No. 357] of 2005), establishes discharge parameters for polluting sources (industry and domestic wastewater) that dump their liquid waste in superficial water bodies at the national level. The maximum permissible value for surfactants was established as 2 mg/L.

In Chile, the National Commission for the Environment, Ministry of Public Works, and Ministry of the General Secretary of the

Presidency issued regulations for the discharge of various pollutants, including detergents (MBAS), into water bodies, establishing maximum permissible levels of 10 mg/L in lake waters.

In Canada, NPEOn and nonylphenols are regulated substances, with maximum allowable values of 0.025 and 0.0025 mg/L, respectively. In Australia, following the guidelines established by the Environmental Protection and Heritage Council and the Department of Energy (Canadian Council of Ministers of the Environment) 543 Utilities and Sustainability, largely employed commercial detergent powder containing methylene blue active substances (MBAS) are of utmost importance, with a maximum allowable limit of 50 mg/L. In general, Brazil, Chile, and Colombia have more flexible regulations (20 mg/L) than those established in other countries (2 mg/L) in terms of the presence of anionic detergents in water, indicating a high toxicological risk to biota, including humans.

CONCLUSIONS

The nonylphenols and NPEOn are emerging pollutants, potent endocrine disruptors, and ecotoxins. The main toxicity mechanisms include the alteration of cellular redox homeostasis (Dwivedi et al., 2022). These contaminants have been detected in different environmental matrices (Ringbeck et al., 2021). They are used for producing nonionic ethoxylates and other widely used chemicals in industries and homes, in textiles, cleaning agents, pesticide formulations, paints, and laundry cleaners (Hong et al., 2020). Moreover, it has been reported that these compounds can be persistently detected in the environment and exert endocrine effects on humans and biota, in both invertebrate and vertebrate animals. In humans, their main effects include endocrine disruption, contamination, feminization of aquatic organisms, allergic reactions, bioaccumulation, mutations, and cancer induction, but cancer has also been reported in different biological models, mainly aquatic organisms (Noorimotlagh et al., 2020). Hence, it is critical to establish relevant legislation regarding the use of these pollutants and their effects on biota, humans, and the environment.

Acknowledgments—The authors are grateful to the National Program for Doctoral Formation-Minciencias for grant support (727–2015. Res. 513, July 2015) and the University of Cartagena, Environmental and Computational Chemistry Research group (2016–2020).

Disclaimer—The authors declare no conflict of interest.

Author Contributions Statement—**Ana C. De la Parra-Guerra**: Conceptualization; Investigation; Visualization; Writing—original draft and review & editing. **Rosa Acevedo-Barrios**: Conceptualization; Investigation; Visualization; Writing—original draft and review & editing.

Data Availability Statement—Due to the nature of the research, the data supporting the findings of our study are available within the article.

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